Remarks

In the specification, paragraphs on pages 9 and 11 were amended to correct minor editorial problems.

Claims 1-23 were pending in the present application. No claims were cancelled. Claims 24-29 were added. Claims 1-4, 10-13, 16, and 20-23 were amended. Therefore, claims 1-29 are now pending.

Support for the new and amended claims can be found throughout the specification, for example:

Claims 1, 13, 22, and 23: page 7, lines 10-13 and 18-23.
Claims 1 and 13: page 7, lines 24-26.
Claims 2 and 3: claim 1; amended to clarify antecedent basis.
Claims 4, 10, 16, 22 and 23; amended to remove unnecessary claim language.
Claims 10, 11, 20 and 21: amended to clarify antecedent basis.
Claim 12: page 17, lines 4-6.
Claims 13 and 23 were amended to clarify the claims.
Claims 24 and 25: page 7, lines 24-26.
Claims 25-28: page 9, lines 13-19.
Claim 29: page 7, lines 24-26; page 9, lines 13-19, and page 17, lines 4-6.

None of the amendments made herein is intended to narrow the claims since none of the amendments were made in view of prior art.

Applicants thank Examiner Hines for the courtesy of an interview with Applicants' representative Sheree Lynn Rybak, Ph.D. on August 7, 2003. During this telephone interview, the following rejections were discussed.

In order to overcome the 35 U.S.C. § 112, first paragraph rejection of claims 11 and 21, Examiner Hines suggested that the Jodar *et al.* document (*Vaccine*, 21:3265-72, 2003) which

demonstrates that detection of functional antibody using the claimed method indicates the efficacy of the vaccine, be described and submitted in the form of a Rule 132 Declaration.

Applicants' representative presented alternative claim language to overcome the 35 U.S.C. § 112, second paragraph rejections. It was agreed that the following amendments would overcome the rejections: claims 1, 13, 22 and 23 would be amended to remove the phrase "and distinguishing" and change the term "containing" to "with;" claims 2 and 3 would be amended to remove the term "first;" claims 4 and 16 would be amended to remove the term "derived;" claims 10 and 20 would be amended to remove the term "vaccine;" and claim 22 would be amended to remove the term "standard."

Lastly, the 35 U.S.C. § 103 rejection was discussed. Applicants' representative explained that the method disclosed in the present application allows one to analyze several different serotypes simultaneously. In contrast, in the methods of the prior art each serotype was analyzed separately because of the cross-reactivity observed. Examiner Hines indicated that this rejection could be overcome by presenting references that explain why it would not be obvious to analyze multiple serotypes at the same time.

Oath/Declaration

As requested by the Examiner, two new declarations are submitted herein (one signed by inventor Hickey, the other signed by the remaining inventors). The new declarations do not contain any non-initialed or non-dated alterations, and identifies the residence for all inventors.

Therefore, applicants request that the objection to the declaration be withdrawn.

Specification

As requested by the Examiner, the specification has been amended on pages 9 and 11 to note the generic terminology for TEXAS RED, EDANS and BODIPY. In addition, the term "Texas Red" was capitalized because it is a registered trademark.

In view of these amendments, Applicants request that the objection to the specification be withdrawn.

35 U.S.C. § 112, first paragraph

Claims 11 and 21 were rejected under 35 U.S.C. § 112, first paragraph on the ground that these claims contain subject matter that was not enabled in the specification. Applicants respectfully disagree and request reconsideration. As discussed in the enclosed Rule 132 Declaration, Jodar *et al.* (*Vaccine*, 21:3265-72, 2003) demonstrate the ability of the claimed method to indicate the efficacy of a vaccine. The vaccine tested, PREVNAR, is a licensed vaccine that contains seven different *Streptococcus pneumoniae* serotypes. The clinical efficacy of PREVNAR is known, as it is approved for use in infants as part of their routine vaccination schedule. Figure 1 of Jodar *et al.*, shows the aggregation of results of multiple ELISA assays of 30,000 subjects vaccinated with PREVNAR. The results in Figure 1 demonstrate that of those subjects vaccinated who achieved an antibody titer of 0.20 μg/ml, 97.9% are disease free, while subjects not vaccinated or those not achieving an antibody titer of 0.20 μg/ml have a 12.9% chance of getting a *Streptococcus pneumoniae* infection.

These efficacy results of PREVNAR using the ELISA method were confirmed using the opsonophagocytic assay disclosed in the present application. As shown in Figure 2 of Jodar *et al.*, the presently claimed opsonophagocytic assay can discriminate between those protected by the vaccine and those who are not protected. The results shown in Figure 2 demonstrate that there is a correlation between the 0.2 μg/ml threshold antibody concentration obtained using ELISA (Figure 1) and the threshold opsonic antibody titer of 1:8 obtained using the opsonophagocytic assay of the present invention (Figure 2). Therefore, the method disclosed in the present application was shown in Jodar *et al.* to indicate the efficacy of the PREVNAR vaccine.

Because the results shown in Jodar *et al.* demonstrate that vaccine efficacy can be demonstrated using the opsonophagocytic assay disclosed in the present application, Applicants request that the 35 U.S.C. § 112, first paragraph rejection be withdrawn.

35 U.S.C. § 112, second paragraph

Claims 1-23 were rejected under 35 U.S.C. § 112, second paragraph on the ground that the claims are indefinite.

Claims 1, 13, 22, and 23 were amended to delete the phrase "and distinguishing" to clarify the claims.

Claims 1, 13, 22, and 23 were amended to change the word "containing" to the word "with" in order to clarify what the sample is being combined with.

Claims 2 and 3 were amended to remove the word "first" in order to clarify the antecedent basis.

Claims 4 and 16 were amended to remove the word "derived" to clarify the claims.

Claims 10 and 20 were amended to remove the unnecessary claim language "a vaccine containing" in order to clarify the claims.

Claim 22 was amended to remove the word "standard" to clarify the claim.

In view of these amendments, Applicants request that the 35 U.S.C. § 112, second paragraph on the ground be withdrawn.

35 U.S.C. § 103(a)

Claims 1-10, 12-20 and 22-23 were rejected under 35 U.S.C. § 103(a) on the ground that these claims are obvious over Romero-Steiner *et al.* (*Clin. Diagn. Lab. Immunol.* 4:415-22, 1997) in view of Sveum *et al.* (*J. Immunol. Meth.* 90:257-64, 1986). Applicants respectfully disagree and request reconsideration.

The present application is directed to a method that allows one to determine opsonophagocytic activity *simultaneously* against *multiple* bacterial serotypes. Previous methods used to detect serotypes present in a sample were limited because they only allowed detection of functional antibodies for each bacterial serotype, one at a time. For example, Concepcion and Frasch (*Clin. Diag. Lab. Immuno.* 8:266-72, 2001; copy enclosed) describe the use of an ELISA based assay to quantitate IgG levels in a subject immunized with multiple *Streptococcus pneumoniae* serotypes. As noted in the abstract, cross-reacting antibodies present in sera decrease the specificity of the results. This cross-reactivity likely occurs because polysaccharides present on the antibodies are similar. Therefore, those skilled in the art would expect that multiple serotypes could *not* be analyzed simultaneously, due to this cross-reactivity between antibodies.

Because of this cross-reactivity, previous assays only permitted analysis of a single serotype at a time. In order to analyze multiple serotypes, large numbers of samples needed to be prepared and analyzed. As noted in Concepcion and Frasch (page 266, second column), opsonophagocytosis assays were not preferred by some investigators because they were labor-

intensive and difficult to perform with large number of samples. Large numbers of samples were required because only one serotype could be analyzed at a time.

Another type of assay commonly used to analyze antibodies produced to bacterial serotypes is the standardized serum bactericidal assay (SBA). However, like the ELISA and previous opsonophagocytic assays, SBA only permits analysis of a single serotype at a time (Mountzouros and Howell, *J. Clin. Microbiol.*, 38:2878-84, 2000). As discussed on page 2878, first column of Mountzouros and Howell, the SBA can only measure a single characteristic of a specific-specific subclass of immunoglobulins. In addition, the method is considered labor intensive and results highly dependent on the source of complement. Therefore, the present method is an improvement over the SBA art because it allows one to *simultaneously* measure functional antibodies against different serotypes (see Martinez *et al.*, *Clin. Diagn. Lab. Immunol.* 9:485-8, 2002; page 486, column 2).

In summary, although it is concluded in the Office action that those skilled in the art would be motivated to use a multiplexed assay to simultaneously detect multiple bacterial serotypes, this is not the case here because the art teaches that there was not a reasonable expectation of success, due to the cross-reactivity of antibodies.

Romero-Steiner *et al.* teach an opsonophagocytic assay that uses HL-60 cells to determine if functional antibodies for seven different pneumococcal serotypes are present in a sample obtained from a vaccinated subject. However, unlike the method disclosed in the present application, the Romero-Steiner *et al.* method can only determine the presence of a *single* serotype at a time. That is, in the Romero-Steiner *et al.* method, a different reaction must be performed for each serotype to be analyzed. In order to determine if antibodies recognizing each of the seven serotypes, seven different reactions must be performed and each analyzed separately. In contrast, the inventors' method allows one to *simultaneously* determine if *multiple* antibodies, each recognizing one of multiple bacterial serotypes, are present in a sample.

Sveum *et al.* teach a two-color fluorescent method for quantitating adherence and ingestion of *S. pneumoniae* by human monocytes. Although the method of Sveum *et al.* uses two different fluorophores, it does not teach or suggest simultaneously detecting multiple bacterial serotypes. In fact, it does not teach detecting bacterial serotypes at all. Instead, the method allows one to detect the presence of ingested bacteria themselves.

It is concluded in the Office action that one skilled in the art would be motivated to use an alternative and functionally equivalent labeling system, such as using labeled beads or labeled antigens. As discussed above however, those skilled in the art would not be motivated to use a multiplexed assay to simultaneously detect multiple bacterial serotypes, because the art teaches that there was not a reasonable expectation of success due to the cross-reactivity of antibodies.

In summary, those skilled in the art would not have expected that a method that permitted simultaneous detection of multiple serotypes would work, due to the known cross-reactivity between antibodies.

If any matters remain before a notice of allowance is issued, the examiner is invited to telephone the undersigned.

Respectfully submitted,

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